

Sterile Matings and Fertility in  
Female Voles, with special  
reference to *Clethrionomys glareolus*

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1983

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*Harvard Law:*

*Under the most rigorously controlled conditions of pressure, temperature, volume, humidity, and other variables, the organism will do as it damn well pleases.*

*Murphy*

**STERILE MATINGS AND FERTILITY IN FEMALE VOLEs, WITH  
SPECIAL REFERENCE TO *CLETHRIONOMYS GLAREOLUS***

**Doctoral thesis**

**by**

**Fil kand Lilian M Westlin**

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**Title and subtitle**

Sterile Matings and Fertility in Female Voles, with special reference to *Clethrionomys glareolus*.

**Abstract**

Sterile matings initiate the breeding season in several microtines. The corpora lutea (CL) resulting from these matings differ from those of pregnancy in that they are smaller, their cells are less luteinized, and they have a core of connective tissue. Fertility is related to age in the bank vole, *Clethrionomys glareolus*: the older the female, the higher the fertility at the first mating. Treatment with progesterone or prolactin shortly after mating dramatically increased fertility. Progesterone treatment results in 100% fertility and prolactin activates the CL resulting in a substantially increased fertility. Females previously kept with vasectomized males showed a much higher fertility than females previously kept a) singly, b) in pairs or c) with a male, but separated from him by a wire mesh. Furthermore, the proportion of matings with fertile males was greatly increased in the females kept with vasectomized males compared to the females kept according to a) or b) and slightly higher than the females in c). This experiment shows that sexual experience highly increases the activation of the luteotrophic complex and thus increases fertility. In pubertal females or in females "re-maturing" after a winter anoestrus, the copulatory stimulus received at a normal mating is not sufficient to trigger the luteotrophic complex and pregnancy does not occur. Artificial vaginal stimulation given to pubertal females after a normal mating results in dramatically increased fertility compared to untreated females. Mating thus induces ovulation, but the effect of copulation in stimulating the luteotrophic mechanism is lacking. The CL resulting from sterile matings have a short life span. Six days after mating (1.5 days after the normal time of implantation) they show almost no enzymatic activity (3 $\beta$ -hydroxysteroid dehydrogenase). However, two days after mating, the enzymatic activity is similar in CL from pregnant and nonpregnant females. This shows that after a sterile mating the CL are in fact functional. Dopamine is a possible prolactin inhibiting factor (PIF) in female bank voles. Daily injections with a dopamine antagonist (haloperidol) in newly mated females resulted in full activity of the luteotrophic complex. This thesis shows that sterile matings in the bank vole have a puberty-accelerating function with a complex mechanism, including both internal and external factors. It is possible that what is regarded as pregnancy block in wild voles, more likely is an effect of immaturity of the mechanism activating the luteotrophic complex.

**Key words**

Bank voles, Reproduction, Sterile matings, Corpora lutea, Reproductive hormones, Fertility, Puberty-acceleration, Pregnancy block

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**Signature** Lilian Westlin **Date** 1983 03 01

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## INTRODUCTION

Bank voles are, like other microtines, reflex ovulators, i.e. the coitus triggers ovulation (Breed, 1967; Clarke, Clulow & Greig, 1970; Milligan, 1975; I). After ovulation, any of three events follows: pregnancy, pseudopregnancy or non-pregnancy. This thesis deals mainly with the last matter.

Following ovulation, the follicle converts to a corpus luteum (CL) under the influence of a luteotrophic complex. During the preimplantation stages of pregnancy, prolactin from the pituitary is known to maintain the CL in rats (Freeman, Smith, Nazian & Neill, 1974), mice (Choudary & Greenwald, 1969) and the vole *Microtus agrestis* (Milligan, 1975). If, for some reason, the luteotrophic support is not sufficient, the CL will regress. In spontaneous ovulators, like rats and mice, the CL of the oestrus cycle will regress if no mating occurs (Conaway, 1971; Freeman et al., 1974) and mating also stimulates luteal function in the reflex ovulating microtine rodents (Milligan, 1975; Kenney & Dewsbury, 1977; Andersson & Gustafsson, 1982). In field voles (Milligan, op. cit.) and in bank voles (Andersson & Gustafsson, op. cit.) mating activates two neuroendocrine reflex mechanisms which separately induce ovulation and the development of luteal function. Both mechanisms are normally activated by an unrestricted mating, while a limited mating stimulates ovulation but no functional luteal phase follows.

It has been claimed that "since wild female microtines normally mate when they enter into full estrous, a complete non-pregnant cycle is rare in nature" (Conaway, 1971) "and thus would not be detected histologically in wild-trapped animals" (Hasler, 1975). It has also been said that "in a wild population a non-pregnant female is either juvenile, senile or a failure, and mechanisms must operate to safeguard the species against any of these occurring too frequently" (Weir & Rowlands, 1973).

However, during field studies we found that at the beginning of their breeding season all trapped female bank voles, comprising both overwintered and young of the year, had experienced several ovulations without becoming pregnant (I). This was disclosed at histological studies of the ovaries. Several sets of CL could be distinguished, also in the specimens which at the time of trapping were pregnant. The literature revealed that the phenomenon of sterile ovulations was quite common both in spontaneous ovulators like *Peromyscus maniculatus* and *P. boyleyi* (Jameson, 1953), *P. eremicus*, *Mesocricetus auratus*, *Rattus conatus*, *Ondatra zibethica* (Asdell, 1964) and several others, and in various induced ovulators, e.g. *Clethrionomys rutilus* and *Microtus oeconomus rattiiceps* (Hoyte, 1955), *M. californicus* (Greenwald, 1956), *M. pinetorum* (Kirkpatrick & Valentine, 1970), *M. pennsylvanicus* (Mallory & Clulow, 1977), *M. ochrogaster* (Rose & Gaines, 1978). Brambell and Rowlands observed already in 1936 that the bank vole had sterile "cycles", believing the species to be a spontaneous ovulator.

The aim of this thesis was to investigate:

- a) the causes of the sterile ovulations in *Clethrionomys glareolus*,
- b) the underlying mechanisms,

- c) the occurrence of the phenomenon in other microtines and
- d) the biological significance of sterile ovulations.

## MATERIAL AND METHODS IN GENERAL

### Field studies

The animals were captured in snap-traps. Immediately after trapping they were dissected, weighed and the condition of the mammary gland, vaginal opening, and the presence or absence of embryos were noted. The reproductive organs were removed, weighed fresh, and fixed in Bouin's fluid. The first mandibular molar,  $M_1$ , was used for age classification (Pucek & Zejda, 1968). All organs were routinely treated for histological studies (I; II).

### Laboratory experiments

I used female bank voles, *Clethrionomys glareolus*, from a colony originating from animals trapped in Skåne, southern Sweden, between autumn 1974 and spring 1975. The colony is maintained at the Department of Zoology, University of Lund, Sweden and is described by Gustafsson, Andersson and Westlin (1980).

After weaning, at 18 days of age, the females were kept in single sex groups until the start of the experiment. Since my interest was focused on the onset of breeding, I started the experiments when the females were 30 days old, except in paper I where the age was 23-43 days. Puberty occurs in this species at about 35 days of age (Sviridenko, 1959).

All the laboratory experiments (except in paper IV) started with mating tests (for description, see the separate papers). After mating, the treatments started. The different treatments are thoroughly described and motivated in each paper and will only be briefly mentioned here.

They were:

- a) Hormone treatment; progesterone or prolactin (Paper III).
- b) Additional artificial vaginal stimulation with a vibrating plastic rod (Paper V).
- c) Treatment with a dopamine antagonist (haloperidol) (Paper VII).
- d) In paper IV the treatments preceded the mating procedure and consisted of different environmental conditions between 30 and 40 days of age.
- e) Paper VI deals with the question whether the CL resulting from sterile matings possibly have any function. This paper is a histochemical study of enzymatic activity in the CL. The studied enzyme,  $3\beta$ -hydroxysteroid dehydrogenase, is required for all steroid production.

All females were autopsied 6 days after mating and checked for pregnancy. The reproductive organs were dissected out and treated for routine histological studies.



## RESULTS AND DISCUSSION

Previous studies on several microtines claim that sterile ovulations occur rather sporadically in nature. The findings that *Clethrionomys glareolus* (I), *Microtus agrestis* (II) and *C. rufocanus* (II) as well as several other microtines (for references, see papers I, II) mate without becoming pregnant at the onset of the breeding season suggest instead that this may be a general phenomenon in microtines.

For the three species mentioned above the infertility has been proved not to be due to 'ineffective' males but to temporary sterility of the females (I; II).

Several mating tests have revealed that following mating all female bank voles possess CL, confirming that the infertile matings are not caused by failure of ovulation (I; III).

The histological studies of the ovaries from mated non-pregnant females revealed that the CL differed from those of pregnant specimens (I; II). They were smaller, less vascularized and the cells seemed poorly luteinized and contained pyknotic nuclei (see paper I: fig. 1 and paper II: figs. 1 and 4). The histology of the CL indicated endocrine activity only for a short time after mating (1-3 days) (I); four - five days after mating the CL had clearly started to regress. It seemed unlikely that these CL could produce enough progesterone to maintain pregnancy. Pregnancy in mammals is established by the hormonal activity of the CL, as its secretion (progesterone) enables the endometrium to accept the early blastocyst for implantation (Weir & Rowlands, 1973).

I injected newly mated females with progesterone once a day for five days and at autopsy all treated females were pregnant (III). Evidently the infertility following mating is not the result of any incompetence in the gametes following induced ovulation or failure of fertilization, but is rather due to the lack of progesterone from the short-lived CL (III). The progesterone treatment did, however, not stimulate the development of luteal function: the proportion of pregnant females with healthy CL did not differ from the controls (see paper III: Table 1). This indicates that progesterone feedback alone is not sufficient to stimulate the luteotrophic system (II).

Milligan's work (1975) on *Microtus agrestis* has shown that, as in rats (Freeman et al., 1974) and mice (Choudary & Greenwald, 1969), prolactin is luteotrophic. This influenced me to investigate whether this is true also for the bank vole. I injected three different doses of prolactin into newly mated females every 12th hour for five days and the response was dose-dependent: the higher the dose of prolactin injected, the higher the fertility rate (III). The dose-dependency indicates that the amount of luteotrophic stimuli required for maintaining the CL may be individual (III). There are also species differences in the dose needed for CL activation. Bank voles seem to require a higher dose (8 i.u./day) than field voles (*Microtus agrestis*), in which 0.5 i.u./day is sufficient to activate the CL (III; Charlton, Milligan & Versi, 1978). The experiments with progesterone and prolactin



confirmed that the cause of infertility in young mated female bank voles was a lack of luteotrophic support (I; III).

The sterile matings occurring in young females and at the start of the breeding season in the field (I) reflect the ease with which mating induces ovulation, but also the relative lack of effect of copulation in stimulating the luteotrophic mechanism (I; III).

The mechanisms underlying the regulation of the luteotrophic complex are still not fully understood. Many hypotheses have been presented through the years. The results of my experiments have made me focus on two factors:

a) coitus and b) the regulation of prolactin secretion.

In induced ovulators, coitus is followed by an LH-surge which causes ovulation. About 20 minutes after mating there is a short small peak of prolactin. After that the level of prolactin successively increases to a peak level around the time for implantation (Spies & Niswender, 1971). It is well known that gonadotropin secretion from the anterior pituitary in mammals is under neural control and that the hypothalamus constitutes the main control center (Harris, 1955; 1972). There is little doubt that anterior connections of the hypothalamus with the other parts of the brain are of the greatest importance in the control of ovarian function (Donovan, 1970). Köves and Halász (1970) have presented evidence that the preoptic area itself may be the origin of the neurogenic stimuli that cause the release of LRF and FRF in amounts necessary for ovulation.

Several studies have shown that extrahypothalamic structures can influence pituitary-ovarian function (Everett, 1964; Kalra et al., 1971; Clemens et al., 1971; Tindal et al., 1967; Carrer & Taleisnik, 1970; Flerkó & Bárdos, 1966).

Hypothalamic and extrahypothalamic mechanisms thus seem to exert a major control function over the secretion of FSH, LH and prolactin from the anterior pituitary. Inputs from the sense organs and from the reproductive tract may be involved in triggering or timing the events of the neural control system. Bilateral resection of the pelvic nerve in rats prior to mating blocks pregnancy and pseudopregnancy by preventing sustained luteal function (Spies & Niswender, 1971). Since luteal activation occurred in intact, but not in pelvic-neurectomized rats, the increase of serum levels of FSH, LH and prolactin at 20 minutes post coitus is not important in initiation of CL function. In contrast, the marked increase in serum prolactin at 8 - 24 hours after mating in intact females may be of major importance in luteal activation (Spies & Niswender, op. cit.). Recent work by Bethea and Neill (1980) showed that stimulation of the uterine cervix (CS) induced two daily surges of prolactin in rats. Bilateral lesions of the suprachiasmatic nucleus completely abolished these surges. Similar results were also shown by Kawakami and Arita (1981). These studies show that the stimulation that the cervix receives at coitus mediates the activation of the luteotrophic complex in rats.

Maybe the stimulation received at the first coitus is not enough to fully activate the luteotrophic complex in bank voles?

This hypothesis is strengthened by the work of Milligan (1975) and Andersson and Gustafsson (1982) on *Microtus agrestis* and *Clethrionomys glareolus* respectively. Adult females require only limited mating (1-3 intromissions) to ovulate but an unrestricted mating to further activate the luteotrophic complex. Ovulation and development of luteal function are in these species induced by two separate neuroendocrine reflex mechanisms, which are activated by mating. The following experiment revealed that the assumption was right. Additional artificial vaginal stimulation with a vibrating rod after mating increased fertility in the treated females, and they developed large active CL (V).

The next step was to find out whether environmental factors prior to the first mating with a fertile male could influence fertility in the female. Between 30-40 days of age, females were kept A) singly, B) in pairs, C) with a male but separated from him by a wire mesh or D) with a vasectomized male. From day 41 onwards each female was paired with an intact male. Females previously kept with a vasectomized male (D) differed significantly from the rest of the females (see IV): a much higher proportion of females mating with the fertile male was found in this group and the age at which this mating occurred was lower. More interesting was that fertility was also improved, and that within the group a difference could be seen between those which had mated with the vasectomized male ( $D_M$ ) and those which had not ( $D_U$ ). The  $D_M$  females showed a much higher fertility at the subsequent mating with the intact male. Furthermore, those  $D_M$  females which had mated twice with the vasectomized male showed a higher fertility than those which had mated only once. From these results we draw the conclusion that sterile matings accelerate sexual maturation in young female bank voles (IV).

Since sterile matings are also found in adult overwintered females (I), and since there are strong resemblances between "re-maturing" at the beginning of the breeding season and puberty (I), there are good reasons to believe that the infertility has similar causes (V) and also a similar function as in young females (IV).

The next question that arose was: how is the prolactin secretion regulated? This is discussed by many researchers.

The hypothalamus inhibits the release of prolactin by a prolactin-inhibiting factor (PIF). There is also evidence of a prolactin-releasing factor (PRF) (Meites et al., 1960; Mishkinsky et al., 1968; Nicoll et al., 1970). The factors have been located in several areas of the hypothalamic region (Pasteels, 1970; Krulich et al., 1971) and PIF activity also in rat hypophysial portal blood (Fink et al., 1967; Kamberi et al., 1970). There is a tuberoinfundibular dopamine neuron system which ends in the median eminence (Fuxe & Hökfelt, 1969, 1970; Shute, 1970). This system may be involved in the transmission of information from the brain to the pituitary. The dopamine neurons may act to control the release of FRF, LRH and PIF from the secretory neurons (see McCann, 1970; Fuxe & Hökfelt, 1969, 1970). It has been shown by several researchers (VanMaanen & Smelik, 1968; MacLeod, 1969; Birge, Jacobs, Hammer & Daughaday, 1970; MacLeod,



Fontham & Lehmeier, 1970; Shaar & Clemens, 1974) that dopamine inhibits prolactin release. Furthermore, Müller, Bauknecht and Siebers (1980) showed that in rats treated with a dopamine agonist, bromocriptine, implantation did not occur. The failure to implant was probably the consequence of changes of the uterine mucosa caused by the lack of progesterone production in the luteal cells (Müller et al., op. cit.).

All these studies inspired me to investigate whether dopamine may act as a PIF in female bank voles. Administration of a dopamine antagonist (haloperidol) in newly mated females activated the luteotrophic complex in all specimens and I therefore suggest that dopamine is a PIF in female bank voles (VII). Also, fertility was greatly increased.

There is no longer any doubt that sterile matings are a puberty phenomenon and not a "failure" of nature. This is also supported by the fact that sterile matings are related to age: fertility increases from less than 25 % in females younger than 50 days to approximately 80 % in 120-128-day-old females (see paper V: fig. 1).

In order to explain, if possible, the puberty accelerating effect of sterile matings, I assumed that the CL formed after a sterile mating must have some possibility to produce progesterone which may act on the uteri. In an experiment (VI) we unilaterally ovariectomized females two days after mating. Four days later they were autopsied, checked for pregnancy and the remaining ovary was removed. All ovaries were treated in a similar way for enzyme-histochemical studies. We wanted to compare the enzymatic activity necessary for steroid production ( $3\beta$ -hydroxysteroid dehydrogenase) between nonpregnant and pregnant females prior to and after the time of implantation. Implantation occurs about 4.5 days after mating in bank voles (Åndersson & Gustafsson, 1979). Six days after mating there were, as expected, large differences between pregnant and nonpregnant females: a high enzymatic activity in the former group and almost no activity in the latter ones (see paper VI: plate 1). Two days after mating, there were no visible differences in the strength of reaction for the enzyme  $3\beta$ -HSD between the pregnant and nonpregnant animals. This suggests that the CL resulting from sterile matings - like those of pregnancy - produce progesterone at this time.

It has been speculated that, at the onset of the breeding season in seasonal breeders, a situation similar to that of puberty prevails (Zuckerman & Weir, 1977). This is supported by the fact that, after a winter anoestrus, trapped female bank voles had all experienced sterile matings (I). There is an inhibition of the release of gonadotropin during sexual quiescence which in some way is altered by light and/or other external environmental stimuli (Donovan, 1967) resulting in altered levels or ratios of gonadotropins. There may be changes in ovarian sensitivity to gonadotropins and pituitary sensitivity to releasing factors. It therefore seems plausible that the infertility of pubertal females and of adult females after a winter anoestrus has similar causes.

Sterile ovulations have been regarded as evidence for occurrence of pregnancy block (Bruce, 1959) in natural populations (Clarke & Clulow, 1973; Clulow & Mallory, 1974; Mallory & Clulow, 1977). The results in this thesis indicate that the occurrence of sterile matings in "re-maturing" or pubertal wild bank voles may be explained by immaturity of the mechanism activating the luteotrophic complex, rather than by pregnancy block (III; IV and especially V).

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# Sterile matings initiate the breeding season in the bank vole, *Clethrionomys glareolus*. A field and laboratory study

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The start of the breeding season of overwintered female bank voles, *Clethrionomys glareolus*, and also the start of breeding in young of the year, was characterized by a general occurrence of sterile ovulations. Similarly, in laboratory experiments, 23 out of 42 young virgin females failed to become pregnant after the first mating, and often mated several times before becoming pregnant. The experiments indicated that the sterile ovulations were attributed to temporary sterility of the females during maturation either at adolescence or at the beginning of the new breeding season, and not a result of (1) initial ovulations of the season being spontaneous, (2) male sterility, or (3) male-induced pregnancy blockage.

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Le début de la saison de reproduction chez le campagnol roussâtre *Clethrionomys glareolus*, se caractérise par des ovulations stériles, et chez les femelles qui ont survécu à l'hiver, et chez les nouvelles femelles reproductrices. En laboratoire, le premier accouplement est resté sans fruit chez 23 des 42 femelles vierges et il a souvent fallu plusieurs accouplements avant qu'elles ne deviennent enceintes. Les expériences semblent démontrer que les ovulations stériles sont le résultat de la stérilité temporaire des femelles durant la maturation au cours de l'adolescence ou au début de la saison de reproduction; elles ne seraient donc dues (1) ni à la spontanéité des premières ovulations de la saison, (2) ni à la stérilité des mâles, (3) ni à un blocage déclenché chez les femelles par les mâles.

[Traduit par le journal]

## Introduction

In a study of reproduction in bank voles, *Clethrionomys glareolus*, Brambell and Rowlands (1936) observed that in the spring the females frequently had corpora lutea without being pregnant. Histologically, these corpora lutea were separated into several sets, which the authors thought to represent a corresponding number of spontaneous ovulations. Similarly, Brambell and Hall (1939) considered the vole, *Microtus agrestis*, to be a spontaneous ovulator because overwintered field-trapped animals had corpora lutea from sterile ovulations at the beginning of the breeding season. This was also true for young of the year in the summer. It has now been established that the bank vole (Clarke *et al.* 1970), the vole, *M. agrestis* (Breed 1967) and several other *Microtus* species (see Milligan 1975) are induced ovulators.

Sterile ovulations have also been described in the skomer vole, *C. glareolus skomeriensis*, by Coutts and Rowlands (1969). They suggested that the reason for the sterility might be infertility of the males due to immaturity of the reproductive organs. They observed that in the spring, the accessory reproductive organs were small, though spermatogenesis occurred. The sterile ovulations of the young of the year during the summer, when fertile males certainly were present are, however, difficult to explain in this way. Other species with sterile ovulations at the beginning of the breeding season

include *C. rutilus* and *M. oeconomus ratticeps* (Hoyte 1955), the field mouse, *M. californicus* (Greenwald 1956), the pine vole, *M. pinetorum* (Kirkpatrick and Valentine 1970), *M. pennsylvanicus* (Mallory and Clulow 1977), and *C. rufocanus* (L. M. Westlin, unpublished data).

The present paper concerns the occurrence of sterile ovulations in wild bank voles, and a laboratory study of the nature of such ovulations.

## Material and methods

### Field studies

Bank voles, *Clethrionomys glareolus* Schreber 1780, were collected during the whole breeding season in the Revinge area (south Sweden) 1972-1975, and in Alanäs, Jämtland (north Sweden) in 1973. Trapped animals were weighed and the condition of the mammary gland, vaginal opening, and the presence or absence of embryos were noted. Adrenals, ovaries, and uteri were removed, weighed fresh, and fixed in Bouin's fluid. The first mandibular molar, M<sub>1</sub>, was used for age classification (Pucek and Zejda 1968). For histological examination, the organs were embedded in paraffin, sectioned (6 µm) and stained with Ehrlich's haematoxylin-eosin or Azan. Seventy-four females were examined. For breeding and density characteristics of the populations, see Nyholm and Meurling (1979).

### Laboratory experiments

Seventy-three virgin female and sexually experienced male bank voles from the laboratory colony maintained at the

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Department of Zoology, University of Lund, Sweden, and described by Gustafsson *et al.* (1980) were used. The animals were kept in standard plastic mouse cages (40 cm  $\times$  20 cm  $\times$  15 cm) with a wire mesh cover at a photoperiod of 18 h light: 6 h dark and a temperature of  $22 \pm 5^\circ\text{C}$ . Wood shavings were used as bedding. The animals were allowed water and a commercial mouse food ad libitum, supplemented twice a week with carrots.

After weaning at 16 days of age, the females were housed five per cage until the start of the experiment; they were then 23 to 43 days old. Twelve females were then housed singly in separate cages and served as controls for spontaneous ovulation. Ten other females and 10 males were housed in pairs in large cages (55 cm  $\times$  35 cm  $\times$  20 cm), male and female separated by a wire mesh; these served as controls for ovulations induced by male odour (Clarke and Hellwing 1977). Each of the remaining 51 females was paired with a sexually experienced male. Vaginal smears were taken from all females twice a day at about 9 a.m. and 4 p.m. A copulatory plug or sperm in the vaginal smear was taken as evidence of mating. A recent copulation could also be recognized histologically by the occurrence of sperm in the uteri. The animals were dissected: the control females and those paired females that had not been observed to mate were dissected 45 days after the start of the experiment, and those that had mated several times were examined 7 days after the latest observed mating (pregnancy at that time is easy to discover by the occurrence of small embryos). The females that had been observed to mate only once were dissected at least 7 days after mating.

Ovaries and uteri were weighed fresh, fixed in Bouin's fluid, sectioned at 7  $\mu\text{m}$ , and stained with a tetrachrome combination (Borg *et al.* 1978). They were examined histologically and the females were considered to have ovulated when recently ovulated follicles, corpora lutea, or corpora albicantia were found. The corpora lutea of multiparous females could be divided into different sets on the basis of cellular size and vacuolization, older corpora lutea having smaller and more vacuolized cells.

## Results

### Wild bank voles

Of 57 overwintered females caught at the beginning of the breeding season (to mid-June), 14 were pregnant for the first time, as judged from the absence of placental scars in their uteri, and 43 had ovulated without becoming pregnant. The ovulations were revealed by the occurrence of up to four sets of ovulatory bodies (corpora lutea and (or) corpora albicantia) (Table 1).

The corpora lutea in the nonpregnant females differed from those in the pregnant females in that they were smaller, their cells were less luteinized, and they had a core of connective tissue (Figs. 1a, 1b).

Twelve of the 14 overwintered females that were in their first pregnancy of the year had several sets of ovulatory bodies which were equally well luteinized but could be distinguished by the fact that the corpora lutea of the fertile ovulations were, as in nonpregnant fe-

males, larger than those of earlier, sterile ovulations (Fig. 2). Furthermore, the number of the larger corpora lutea correlated exactly to the number of embryos in the uteri. The absence of zona pellucidum and oocytes in the smaller corpora lutea excluded the possibility that they were accessory corpora lutea. In one of the remaining females corpora albicantia occurred besides the corpora lutea of pregnancy, and in the other the latter type was the only one. Only fertile ovulations occurred in the overwintered specimens which were caught later in the summer.

Of 13 primiparous young of the year that had ovulated in July, 8 were nonpregnant. One to four sets of ovulatory bodies were found in their ovaries (Table 2). The remaining five were pregnant and each had one set of corpora albicantia besides the corpora lutea of pregnancy.

### Laboratory experiments

The 12 control females which were housed separately did not ovulate. Neither did those 10 housed with a male but separated from him by a wire mesh. Of the 51 females that were paired with fertile males, 9 were never observed to mate, and no ovulatory bodies were found. Of the remaining 42 females, 19 (45%) became pregnant after the first observed mating and 23 (55%) did not. Of these latter females, 17 mated more than once; the intervals between the matings were 1–8 days (Table 3).

The corpora lutea of sterile matings were of similar appearance as those found in the nonpregnant wild-caught females. They never reached the size and cellular development of the corpora lutea of pregnancy and connective tissue developed in their residual antrum.

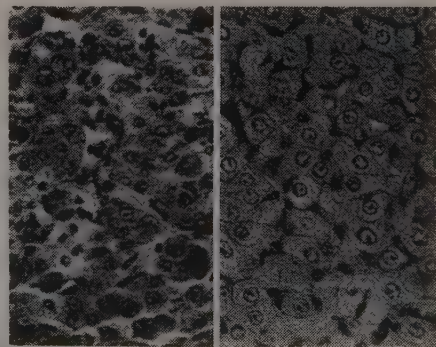


FIG. 1. (a) Corpus luteum from a nonpregnant bank vole. The cells are only poorly luteinized.  $\times 390$ . (b) Corpus luteum of a pregnant bank vole with well luteinized cells.  $\times 390$ .

TABLE 1. Numbers and types of ovulatory body sets in wild overwintered bank voles (*Clethrionomys glareolus*) at the beginning of the breeding season and at the start of reproduction in young of the year

## (A) Nonpregnant, after ovulation

| Number of ovulatory<br>bodies<br>(sets) | Corpora lutea<br>(sets) | Corpora albicantia<br>(sets) | Number of<br>animals |
|-----------------------------------------|-------------------------|------------------------------|----------------------|
| 1                                       | 1                       | 0                            | 6                    |
| 2                                       | 2                       | 0                            | 14                   |
| 2                                       | 1                       | 1                            | 7                    |
| 3                                       | 2                       | 1                            | 13                   |
| 3                                       | 1                       | 2                            | 2                    |
| 4                                       | 2                       | 2                            | 1                    |
|                                         |                         |                              | 43                   |

## (B) Pregnant for the first time

| Number of ovulatory<br>bodies<br>(sets) | Corpora lutea<br>pregnancy<br>(sets) | Corpora lutea<br>reactivated<br>(sets) | Corpora albicantia<br>(sets) | Number of<br>animals |
|-----------------------------------------|--------------------------------------|----------------------------------------|------------------------------|----------------------|
| 1                                       | 1                                    | 0                                      | 0                            | 1                    |
| 2                                       | 1                                    | 1                                      | 0                            | 9                    |
| 2                                       | 1                                    | 0                                      | 1                            | 1                    |
| 3                                       | 1                                    | 2                                      | 0                            | 3                    |
|                                         |                                      |                                        |                              | 14                   |

TABLE 2. Numbers and types of ovulatory body sets in wild, young-of-the-year bank voles (*Clethrionomys glareolus*) at the start of reproduction

## (A) Nonpregnant, after ovulation

| Number of ovulatory<br>bodies<br>(sets) | Corpora lutea<br>(sets) | Corpora albicantia<br>(sets) | Number of<br>animals |
|-----------------------------------------|-------------------------|------------------------------|----------------------|
| 1                                       | 1                       | 0                            | 4                    |
| 2                                       | 1                       | 1                            | 1                    |
| 3                                       | 2                       | 1                            | 2                    |
| 4                                       | 2                       | 2                            | 1                    |
|                                         |                         |                              | 8                    |

## (B) Pregnant for the first time

| Number of ovulatory<br>bodies<br>(sets) | Corpora lutea<br>pregnancy<br>(sets) | Corpora lutea<br>reactivated<br>(sets) | Corpora albicantia<br>(sets) | Number of<br>animals |
|-----------------------------------------|--------------------------------------|----------------------------------------|------------------------------|----------------------|
| 1                                       |                                      |                                        |                              | 0                    |
| 2                                       | 1                                    | 0                                      | 1                            | 5                    |
|                                         |                                      |                                        |                              | 5                    |

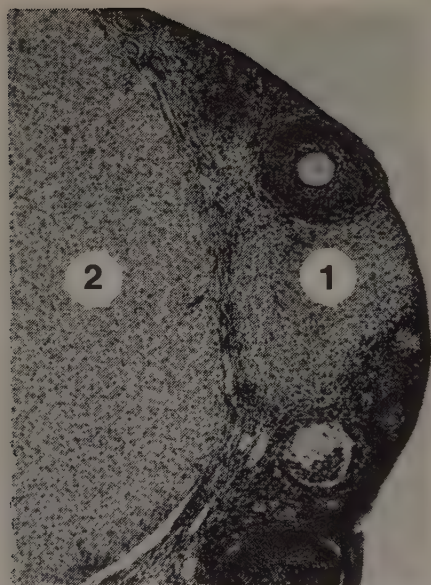


FIG. 2. Ovary from a bank vole, pregnant for the first time. (1) Corpus luteum after a sterile mating, reactivated during formation of (2) corpora lutea after subsequent fertile mating.  $\times 100$ .

The cells showed poor luteinization and degeneration started about 4 or 5 days after mating.

Four of the females became pregnant after a number of sterile matings. In two of them (A), the histological examination of the ovaries revealed corpora albicantia besides the corpora lutea of pregnancy. In the other two (B), two sets of corpora lutea were found, the corpora lutea of pregnancy originating from the last recorded mating (as judged from the size of the embryos) and one set with smaller but equally well luteinized corpora lutea from the penultimate mating, which had obviously been sterile. These smaller corpora lutea were similar to those

described above in some of the pregnant wild voles. In A the interval between the sterile and the fertile mating was 7 days and in B, 3 days.

### Discussion

Previous studies suggest that sterile ovulations occur rather sporadically. However, in our field samples, sterile ovulations appeared to be the general feature both in overwintered animals at the beginning of the breeding season in the spring, and in young of the year during the summer. The difference in frequency of pregnancy after first mating between laboratory and field animals may be due to the environmental differences. For example, the field animals were maturing after winter and probably mostly had access to low quality food, whereas the laboratory animals were well nourished.

In the wild young of the year, the ovulatory bodies, besides the corpora lutea of pregnancy, were all classified as corpora albicantia. However, most of the wild overwintered voles which were pregnant for the first time of the year had two sets of well luteinized corpora lutea. This was never found during later pregnancies. Greenwald (1956) observed a similar phenomenon in *Microtus californicus*, which he ascribed to luteinized follicles (accessory corpora lutea). However, since no remnants of an oocyte or zona pellucidum were found in the "extra" set of corpora lutea in the present material, such a suggestion seems hardly applicable to our study. The development of the "extra" corpora lutea can more probably be explained by the suggestion that the fertile mating occurred so soon after the last sterile mating that the corpora lutea of the latter were still accessible to stimulation to endocrine activity in connection with the subsequent fertile mating. In the laboratory, reactivation of the corpora lutea occurred when the interval between matings was 3 but not 7 days.

Reactivation of corpora lutea of earlier ovulations than those of pregnancy has also been thought to occur in the skomer vole, *C. glareolus skomeriensis* (Coutts and Rowlands 1969).

The level of luteinization of the corpora lutea of sterile matings varied, the older corpora lutea having the less luteinized cells. The histology of the corpora lutea of very recently (1-3 days) mated females indicated that the cells were active and might serve as endocrine tissue, like the corpora lutea of pregnancy of similar age.

The corpora lutea of sterile matings started to degenerate 4 or 5 days after mating. Milligan (1975) observed that limited matings (one intromission) in *Microtus agrestis* resulted in corpora lutea that started to degenerate within 3 days. It seems improbable that the poor developments of the corpora lutea of the sterile ovulations in the present study were caused by incomplete matings, since the pairs were left undisturbed except for the short moments when the females were checked for mating.

TABLE 3. Intervals between successive matings. The interval between successive matings in the laboratory animals varied; 40% of the intervals were 1 or 2 days, but in most cases (75%), 1-4 days

|                  | Length of interval (days) |    |   |   |   |   |   |   |    |    |    |  |
|------------------|---------------------------|----|---|---|---|---|---|---|----|----|----|--|
|                  | 1                         | 2  | 3 | 4 | 5 | 6 | 7 | 8 | 12 | 17 | 22 |  |
| No. of intervals | 7                         | 14 | 9 | 7 | 4 | 3 | 1 | 1 | 1  | 1  | 1  |  |



It has been shown that *Clethrionomys glareolus* is an induced ovulator (Clarke *et al.* 1970). This was confirmed in the present study in that none of the females ovulated when housed separately or when kept in a cage with a wire mesh separating them from the male. Neither did the females that were kept together with a male but did not mate.

Bruce (1959, 1960) showed that pregnancy in laboratory mice (*Mus musculus*) is interrupted if recently inseminated females were exposed to males other than those with which they had mated. Several microtines have similarly been shown to be susceptible to male-induced pregnancy blockage: *M. agrestis* (Clulow and Clarke 1968); *M. pennsylvanicus* (Clulow and Langford 1971; Mallory and Clulow 1977). Mallory and Clulow (1977) suggested that the occurrence of several sets of ovulatory bodies in wild *M. pennsylvanicus* was associated with male-induced pregnancy blockage rather than with adolescent sterility as suggested in *M. californicus* (Greenwald 1956) and *C. glareolus skomeriensis* (Coutts and Rowlands 1969). To be relevant under natural conditions, the pregnancy-blockage hypothesis should imply that the social structure of the vole populations allows sexually receptive females to be exposed to different males at short intervals. To what extent this implication agrees with the actual social conditions in the populations is unknown. Mallory and Clulow (1977) found some evidence of the frequency of the pregnancy failure being related to the population density. Theoretically, increased probability for interactions between individuals might be favoured by increased population densities. However, the present study on *Clethrionomys glareolus* produced no support for an association between pregnancy failure and population density; all the samples of wild females indicated that the breeding season started with a sterile phase. Similar frequencies of sterile matings were found in the northern (cyclic) population in a year of very high spring density (1973) (Nyholm and Meurling 1979) and in the significantly less dense southern populations (1972–1975) (Nyholm and Meurling 1979). It seems more likely that the sterile ovulations were due to temporary sterility of the females, preceding a fertile state. This would agree with the suggestion by Greenwald (1956) and Coutts and Rowlands (1969). The probability of this interpretation was strengthened by the experimental females, which showed initial sterility and development of several sets of ovulatory bodies similar to those in the wild specimens, although they were exposed to only one male.

The ecological significance of sterile matings is unknown and at the present stage of knowledge, speculations seem unjustified.

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# Sterile matings at the beginning of the breeding season in *Clethrionomys rufocanus* and *Microtus agrestis*

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WESTLIN, L. M. 1982. Sterile matings at the beginning of the breeding season in *Clethrionomys rufocanus* and *Microtus agrestis*. Can. J. Zool. 60: 2568-2571.

Female *Clethrionomys rufocanus* and *Microtus agrestis* were captured in Sweden at the beginning of their breeding seasons in 1978 and 1981 and were examined for evidence of sterile matings. Dissection, gross examination, and histological studies revealed that approximately 90% of the captured females had ovulated. These ovulations were most likely caused by mating because all investigated microtines have induced ovulation. All mated females of both species had experienced at least one sterile ovulation. Possible explanations of the phenomenon are discussed. Male *C. rufocanus* captured in the same period did not differ in sexual status from males captured later in the breeding season.

WESTLIN, L. M. 1982. Sterile matings at the beginning of the breeding season in *Clethrionomys rufocanus* and *Microtus agrestis*. Can. J. Zool. 60: 2568-2571.

Femelles de *Clethrionomys rufocanus* et de *Microtus agrestis* ont été capturées en Suède au début de leur saison de reproduction en 1978 et en 1981, afin de déterminer si elles s'accouplent parfois sans résultat. La dissection, l'examen général et l'examen histologique de ces animaux ont révélé qu'environ 90% de ces femelles avaient subi leur ovulation. Ces ovulations ont vraisemblablement été déclenchées par accouplement puisqu'il semble que tous les Microtinae aient une ovulation provoquée. Chez les deux espèces, toutes les femelles accouplées avaient subi au moins une ovulation stérile. Les causes possibles de ce phénomène font l'objet d'une discussion. Les mâles de *C. rufocanus* capturés au cours de la même période avaient le même statut sexuel que des mâles capturés plus tard au cours de la saison de reproduction.

[Traduit par le journal]

## Introduction

In southern and northern Sweden, female bank voles, *Clethrionomys glareolus*, ovulate several times before becoming pregnant at the beginning of the reproductive season (Westlin and Nyholm 1982). These sterile sexual cycles have been described by Brambell and Rowlands (1936) and Coutts and Rowlands (1969) but with the suggestion that this species was a spontaneous ovulator. It has since been shown, however, that *C. glareolus* is an induced ovulator (Clarke et al. 1970; Westlin and Nyholm 1982). In the laboratory, young females usually mate and ovulate several times before becoming pregnant (Westlin and Nyholm 1982). The ovaries of these females show several sets of corpora lutea like those of the field specimens.

Evidence of sterile ovulations has been found in field samples of some other microtines: *Microtus agrestis* (Brambell and Hall 1939), *Microtus pinetorum* (Kirkpatrick and Valentine 1970), *Microtus californicus* (Greenwald 1956), *Microtus oeconomus*, *Clethrionomys rutilus*, and one specimen of *Clethrionomys rufocanus* (Hoyte 1955).

Cumulative evidence suggests that all members of the subfamily Microtinae should be considered as induced ovulators (Clarke et al. 1970; Breed 1967; Milligan 1975) which under certain conditions may ovulate

spontaneously (Hasler 1975). Thus, sterile ovulations are probably preceded by matings. The purpose of the present study was to determine the prevalence of sterile ovulations in *C. rufocanus* and *M. agrestis*.

## Material and methods

In 1978, 21 female and 10 male *C. rufocanus* were captured at the beginning of their breeding season (June 1-10) and 6 males (same species) were captured in the middle of the breeding season (July) in Ammarnäs, northern Sweden.

Thirteen female *M. agrestis* were captured at the beginning of their breeding season in 1981 (which this year occurred in late June (L. M. Westlin, unpublished observations)) in the Revinge area, southern Sweden.

Animals were dissected immediately after capture. The ovaries and uteri were weighed fresh, fixed in Bouin's fluid, sectioned at 7 µm, and stained with a tetrachrome combination according to Borg et al. (1978). The ovaries were examined for corpora lutea and corpora albicantia, which were counted and assigned to different sets, each corresponding to one ovulation. The criteria used for ovulation were the presence of (a) a recently ovulated follicle, (b) a corpus luteum, or (c) a corpus albicans. To ascertain whether the females had been pregnant before capture, the uteri were examined histologically for placental scars.

The reproductive organs of the males were weighed to assess their sexual maturity.

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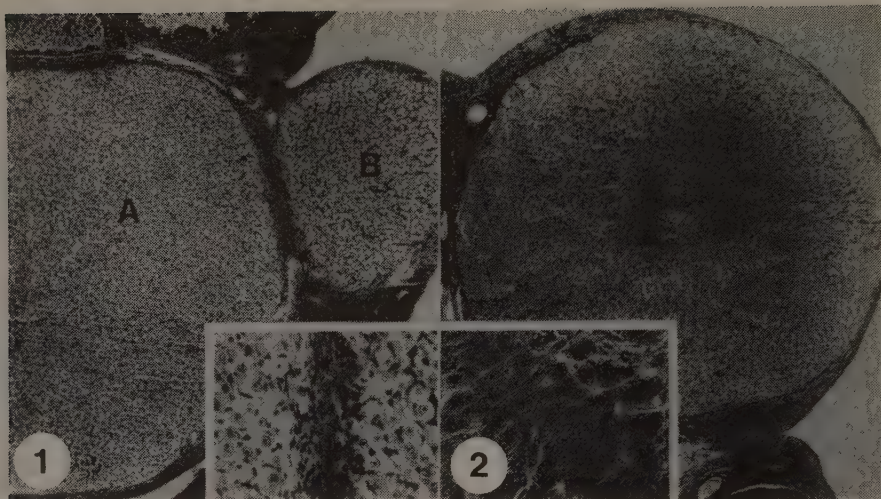


FIG. 1. Part of an ovary of a pregnant *C. rufocanus*. One normal corpus luteum (A) and one older, small, and apparently reactivated corpus luteum (B). 67 $\times$ . Inset: magnification showing equally well luteinized cells in both corpora. 220 $\times$ . The corpora lutea of *C. rufocanus* are much larger than those of *M. agrestis*. FIG. 2. Part of an ovary of a nonpregnant *C. rufocanus* after a sterile ovulation. The corpus luteum has poorly luteinized cells. The absence of pyknotic nuclei, however, indicates that this corpus luteum is in the very beginning of the degeneration process. 67 $\times$ . Inset 220 $\times$ .

### Results

#### *Clethrionomys rufocanus*

All 21 females of this species had perforated vaginas and 19 had ovulated. Among these 19 females, none had recently ovulated follicles, 2 had sperm plugs in the vagina, and all had either corpora lutea or corpora albicantia.

Of the 19 females that had ovulated, 3 were pregnant in a stage beyond implantation. These three had a set of large healthy corpora lutea, the number exactly corresponding to the number of embryos. They also had one or two sets of smaller, reactivated corpora lutea (equally healthy) from recent previous ovulations (Westlin and Nyholm 1982) (Fig. 1) as well as a set of corpora albicantia. The remaining 16 females had ovulated on one or more occasions without becoming pregnant, and their corpora lutea (Fig. 2) histologically resembled those of sterile-mated nonpregnant female bank voles (Westlin and Nyholm 1982). No female had fresh placental scars or was lactating. Thus, the first mating and ovulation of that breeding season did not result in pregnancy in any of the animals.

In 15 of the above 19 females, including the 3 pregnant ones, more than one sterile ovulation had occurred (Table 1).

Three of the nonpregnant females that had sterile ovulations showed old placental scars in their uteri, judging from their small size and pigmentation. They were dated back to the previous breeding season, or, though less likely, to winter breeding. No evidence of winter breeding in this species has been found in the sampling area (E. Nyholm, personal communication).

Judging from their gonads and accessory sex glands the male *C. rufocanus* captured in June 1978 were sexually mature and did not differ in this respect from those captured in July (Table 2).

#### *Microtus agrestis*

Twelve of the 13 captured females had ovulated, and all had perforated vaginas. None of these 12 females had recently ovulated follicles or sperm plugs in the vagina but all had either corpora lutea or corpora albicantia or both.

Seven of the 12 ovulated females were pregnant. They all had more than one set of corpora lutea and (or) corpora albicantia from recent previous ovulations. There was an exact correspondence between the number of embryos and the number of large healthy corpora lutea. The earlier sets of corpora lutea could be distinguished in the same way as in *C. rufocanus*.

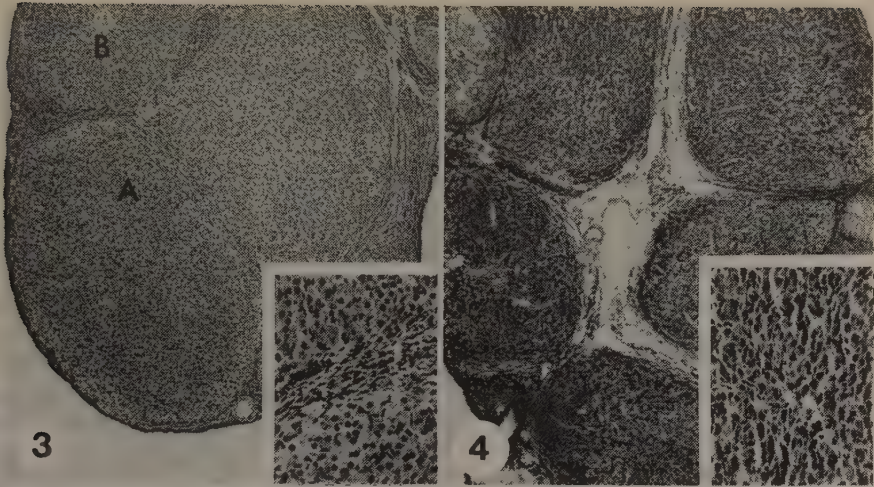


FIG. 3. Part of an ovary of a pregnant *M. agrestis*. One normal corpus luteum (A) and one older, small, and apparently reactivated corpus luteum (B). 67 $\times$ . Inset: magnification showing equally well luteinized cells in both corpora. 220 $\times$ . FIG. 4. Part of an ovary of a nonpregnant *M. agrestis* after a sterile ovulation. The corpus luteum has poorly luteinized cells and is clearly degenerating. 67 $\times$ . Inset 220 $\times$ .

TABLE 1. Distribution of number of sterile matings in female *C. rufocanus* and *M. agrestis* trapped at the beginning of the breeding season. The number within parentheses shows the number of females which became pregnant after a given number of sterile matings

|                                   | No. of sterile matings |      |       |      |      |      |
|-----------------------------------|------------------------|------|-------|------|------|------|
|                                   | 0                      | 1    | 2     | 3    | 4    | 5    |
| No. of female <i>C. rufocanus</i> | 0                      | 4(0) | 10(2) | 3(1) | 1(0) | 1(0) |
| No. of female <i>M. agrestis</i>  | 0                      | 8(4) | 4(3)  | 0    | 0    | 0    |

(Fig. 3). Of the remaining five nonpregnant females, only one had ovulated more than once. These females had corpora lutea (Fig. 4) histologically resembling those seen in the nonpregnant *C. rufocanus*. No female of this species had fresh or old placental scars or was lactating. Thus, such as in *C. rufocanus*, the first mating and ovulation of the breeding season did not result in any pregnancies.

In 4 of the above 12 females, including the 7 pregnant ones, more than one sterile ovulation had occurred (Table 1).

### Discussion

In view of the evidence for induced ovulation in microtines and the occurrence of sterile matings in

TABLE 2. Male *C. rufocanus* captured in June and July (organ weights are given in milligrams  $\pm$  SD)

|      | No. of males | Testes and epididymides | Seminal vesicles  |
|------|--------------|-------------------------|-------------------|
| June | 10           | 918.5 $\pm$ 211.82      | 386.0 $\pm$ 81.65 |
| July | 6            | 812.1 $\pm$ 238.99      | 440.7 $\pm$ 92.17 |

young laboratory-bred *C. glareolus* (Westlin and Nyholm 1982), my findings show that in the beginning of the breeding season both female *C. rufocanus* and *M. agrestis* mate several times before becoming pregnant.

This study may indicate that the number of sterile matings preceding the first fertile one is lower in *M. agrestis* than in *C. rufocanus* (Table 1) or *C. glareolus* (Westlin and Nyholm 1982).

The findings that *M. agrestis* and *C. rufocanus* as well as several other microtines (see Introduction) mate without becoming pregnant at the beginning of the breeding season suggest that this may be a general phenomenon in microtines.

Old placental scars in some specimens of *C. rufocanus* indicated that females that had been pregnant the season before also had sterile matings at the beginning of the following season. In *C. glareolus*, sterile matings have also been noted in young breeding

in their year of birth (Westlin and Nyholm 1982). Thus, the phenomenon does not appear to be related to age or reproductive experience in an previous season in this species. After the first pregnancy of the season, corpora lutea with low activity, as described here, are not seen in either *C. glareolus* or *M. agrestis* (L. M. Westlin, unpublished observations).

Sterile ovulations in the skomer vole, *Clethrionomys glareolus skomeriensis*, in the beginning of the breeding season have been reported by Coutts and Rowlands (1969). These authors suggested that early in the season the males might not be fully sexually mature, with failure of fertilization as a result.

Judging from the weights of testes, epididymides, and seminal vesicles, the male *C. rufocanus* captured in the beginning of the breeding season did not differ in sexual development from those captured in the peak season. In a closely related species, *C. glareolus*, under laboratory conditions the first mating with males known to be fertile are frequently sterile (Westlin and Nyholm 1982).

It is possible that the sterile matings have a priming effect on the female. In microtines the act of mating triggers an ovulatory surge of LH from the anterior pituitary by a neuroendocrine reflex arc. This results in ovulation and after an increase in prolactin level corpora lutea are formed, which are responsible for the secretion of gestational hormones (Milligan and MacKinnon 1976). Repeated matings give rise to a series of corpora lutea (Westlin and Nyholm 1982), i.e., an increasing amount of endocrine tissue. The genital tract may require this sustained stimulation in order to reach maturity and support implantation. Further, according to Gustafsson and Andersson (1980), mating in the bank vole also induces characteristic changes in the adrenal cortex, reflected in a very large increase in weight. The adrenal cortex in this species thus appears to be involved in reproduction, possibly by supplying sex steroids. It might be that in female bank voles the matings in the early part of the season may stimulate endocrine activity in the adrenal cortex.

It appears that the occurrence of regular sterile matings among several small rodents with induced ovulation serves some functional purpose whose nature and significance are still unknown.

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## Increased fertility in young primiparous female bank voles, *Clethrionomys glareolus*, treated with prolactin or progesterone after mating

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**Summary.** Fertility in young primiparous female bank voles was improved by daily injections after mating of progesterone (0.5 mg/day) or prolactin (8 i.u./day). Prolactin treatment increased the number of healthy corpora lutea. The results suggest that the sterile matings at the start of the breeding season are not due to failure of ovulation, but to lack of progesterone from inactive CL.

### Introduction

Female bank voles, *Clethrionomys glareolus*, which are induced ovulators (Clarke, Clulow & Greig, 1970; Westlin & Nyholm, 1982), have several matings at the start of the breeding season that do not result in pregnancy (Westlin & Nyholm, 1982). The corpora lutea (CL) of these sterile matings differ from CL of pregnancy in that they are smaller, less luteinized (as judged from their smaller cell size) and have a short life span (Westlin & Nyholm, 1982). Milligan (1975) found short-lived CL in field voles, *Microtus agrestis*, after limited matings and after hormonally induced ovulations, and that the life span of these CL can be prolonged by exogenous prolactin (Charlton, Milligan & Versi, 1978). In field voles the development of functional CL after mating was associated with elevated prolactin levels immediately following coitus (Milligan & MacKinnon, 1976). Prolactin levels were also elevated 4 days after mating in animals with active CL. The present study was undertaken to examine whether the fertility of young female bank voles could be increased by progesterone treatment (as replacement for the inadequate CL) or by exogenous prolactin (to stimulate the CL).

### Material and Methods

**Animals.** The bank voles, *Clethrionomys glareolus*, were bred in the Department of Zoology, University of Lund, and maintained as described by Gustafsson, Andersson & Westlin (1980).

Females were weaned at 16 days of age and kept in single-sex groups. When 30 days old, the females were isolated, and given mating tests. Males used as studs were >2 months old. The mating procedure was as follows. A male was introduced into the female's cage at 09:00 h, and the female was checked every 2 h for a copulatory plug or for mating behaviour. After mating, the male was removed. If no mating occurred during the day, the male was removed and the test was repeated the following day. Females which had not mated when 2 months old were discarded.

**Injections.** These were given subcutaneously under very light ether anaesthesia beneath the

skin at the back of the head. All injection volumes were 0.1 ml. Progesterone (AB LEO, Helsingborg, Sweden) was made up in sesame oil and administered at a dose of 0.5 mg per injection. Prolactin (ovine prolactin, Sigma Chemical Company) was injected in doses of 0.25, 1 or 4 i.u. per injection. The vehicle was a solution of 3 mg phenol and 50 mg glucose per ml distilled water. Control females were injected with sesame oil only.

**Experimental methods.** After mating the females were randomly assigned to different treatments (see Table 1). Group A was given only sesame oil once a day for 5 days starting 24 h after mating. Group B was given 0.5 mg progesterone/day in the same manner as Group A. In Groups C, D and E prolactin was given every 12 h for 5 days starting 12 h after mating, the total daily dose being 0.5, 2 or 8 i.u. per animal. All animals were killed 6 days after mating, a time when embryos easily can be detected in the uteri as 3–4-mm swellings. At autopsy all ovaries and uteri were examined macroscopically for the presence of large pink CL and embryos respectively, dissected out, fixed in Bouin's fluid, and routinely embedded in paraffin wax. The ovaries were serially sectioned at 7  $\mu$ m and stained with a tetrachrome combination according to Borg, Andersson & Meurling (1978). Histologically, two types of CL were distinguished: (1) large CL with large well luteinized cells and richly vascularized, and (2) small CL with cells containing pyknotic nuclei and degenerated cytoplasm and poorly vascularized (see Westlin & Nyholm, 1982).

### Results and Discussion

The ages at mating were similar in all groups, except in Group B in which most of the females were very young when they mated. No relationship between age at mating and fertility was seen. Following mating, all the females in each group possessed CL, confirming that the infertile matings were not caused by failure of ovulation. In control females (Group A) only a small proportion (35%) of the females became pregnant; the non-pregnant females did not have functional CL. In contrast, progesterone treatment after mating (Group B) resulted in pregnancy in all females, suggesting that the infertility following mating was not the result of any incompetence in the gametes following induced ovulation or failure of fertilization, but rather was due to the lack of progesterone from the short-lived CL. Progesterone treatment itself did not stimulate the development of luteal function: the proportion of pregnant females with healthy CL did not differ from the controls (Table 1). This suggests that progesterone feedback alone is not sufficient to stimulate the luteotrophic system. The results after prolactin administration

**Table 1.** The effect (at Day 6) of daily injections of progesterone and twice daily injections of prolactin on fertility and the function of corpora lutea in mated, primiparous female bank voles

| Group | No. of females | Treatment               | No. of females     |                          |                         |
|-------|----------------|-------------------------|--------------------|--------------------------|-------------------------|
|       |                |                         | With degenerate CL | With healthy CL (%)      | Pregnant (%)            |
| A     | 20             | Sesame oil              | 13                 | 7 (35)                   | 7 (35)                  |
| B     | 20             | Progesterone            | 13                 | 7 (35)                   | 20 (100) <sup>a,c</sup> |
| C     | 15             | Prolactin, 0.5 i.u./day | 10                 | 5 (33)                   | 5 (33)                  |
| D     | 15             | Prolactin, 2 i.u./day   | 7                  | 8 (53)                   | 8 (53)                  |
| E     | 15             | Prolactin, 8 i.u./day   | 1                  | 14 (93) <sup>a,b,c</sup> | 14 (93) <sup>a,c</sup>  |

<sup>a</sup> Different from Group A,  $P < 0.05$ ;  $\chi^2$  test.

<sup>b</sup> Different from Group B,  $P < 0.05$ ;  $\chi^2$  test.

<sup>c</sup> Different from Group C,  $P < 0.05$ ;  $\chi^2$  test.

### Hormones and fertility in young bank voles

(Groups C, D and E) suggest that the short life of the CL may have been due to inadequate luteotrophic stimuli. In the females injected with different doses of prolactin, the frequency of pregnancy increased with increasing dose (Table 1): a significant effect was obtained with 8 i.u./day, a dose that was higher than that, 0.5 i.u./day, which was sufficient to activate the CL in field voles (Charlton *et al.*, 1978).

These results suggest that the sterile matings occurring in young females and at the start of the breeding season in the field (Westlin & Nyholm, 1982) reflect the ease of mating to induce ovulation, but the relative lack of effect of copulation in stimulating the luteotrophic mechanism.

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INFLUENCE OF SEXUAL EXPERIENCE AND SOCIAL ENVIRONMENT ON  
FERTILITY AND INCIDENCE OF MATING IN YOUNG FEMALE  
CLETHRIONOMYS GLAREOLUS

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**Summary.** Between 30 and 40 days of age, female Clethrionomys glareolus were kept either singly, in female pairs, separated from an adult male by a wire mesh, or paired with a vasectomized male. At day 40 they were paired with adult males. Mating with the vasectomized male resulted in high fertility (63%) in subsequent mating with a normal male. In the other treatments, fertility of the first mating was low (22-25%). Thus, sterile matings had a priming effect on the females.

The occurrence of sterile matings in natural populations indicates that such a priming is a part of the puberty process in nature.

In addition to the effect of mating, only 55-59 per cent of the females without contact with males between days 30 and 40 mated with the fertile male. Contact with a male through a wire mesh increased the proportion to 80 and cohabitation with a vasectomized male to 94 per cent. In the last group, mating also took place at a lower age.

## Introduction

It is often assumed that infertile matings are rarely found in wild small mammals (Conaway, 1971). In microtines, however, infertile matings at the beginning of their breeding season may be a general phenomenon. Evidence (in the form of non-functional corpora lutea) has been reported from Microtus agrestis (Brambell and Hall, 1939; Westlin, 1982a), M. pennsylvanicus (Mallory and Clulow, 1977), M. californicus (Greenwald, 1956), M. pinetorum (Kirkpatrick and Valentine, 1970), M. oeconomus ratticeps and Clethrionomys rutilus (Hoyte, 1955), C. rufocanus (Westlin, 1982a), C. glareolus (Westlin and Nyholm, 1982). Some of these authors have attributed non-functional corpora lutea to spontaneous ovulations, but it is now generally accepted that microtines are reflex ovulators (Breed, 1967, 1972; Clarke et al., 1970; Milligan, 1975; Westlin and Nyholm, 1982).

In C. glareolus, fertility of females has been studied more closely. The infertility is not only seen in adult females at the beginning of the breeding season, but also in female young of the year at their first matings (Westlin and Nyholm, 1982). Thus, the infertility may be a puberty phenomenon; the beginning of the breeding season has been compared with puberty (Hutchinson and Sharp, 1977).

Fertility of young female C. glareolus has been studied experimentally. Under laboratory conditions, young females

mated with fully fertile males frequently fail to become pregnant (Westlin and Nyholm, 1982). The infertility is associated with low activity of the corpora lutea and the conceptuses never reach the implantation stage (Westlin and Nyholm, 1982).

Treatment of recently mated females with progesterone increases fertility to 100 per cent without activating the corpora lutea. The activation of corpora lutea is dependent on prolactin: injections of exogenous prolactin result in activation of corpora lutea and dramatically increase fertility (93% compared to 35% in untreated young females) (Westlin, 1982b). It is possible that sterile matings may prime the females and thus increase fertility of subsequent matings.

This work aimed to study this possible priming effect of sterile matings on fertility. The effect of proximity to a male during puberty on fertility was also investigated.

#### Materials and Methods

186 female bank voles (Clethrionomys glareolus) from a laboratory colony kept at the Department of Zoology, University of Lund, Sweden (Gustafsson, Andersson and Westlin, 1980) were used. They were housed in standard plastic cages with wire mesh lids and with wood shavings as litter. They were fed a commercial mouse food supplemented once a week with guinea pig food. The photoperiod was 18L:6D. The females were kept in groups of five from weaning at day 18 until the start of the experiment. Between the ages of 30 and 40 days, they were subjected to four different treatments: A. Housed singly. B. Two females per cage. C. One female and one male per cage, separated by a wire mesh. D. One female and one vasectomized male per cage. The size of the cages was 20 x 40 x 15 cm in groups A, B and D, and 35 x 55 x 20 cm in group C. The females in group D were checked for vaginal plugs twice daily, with approximately 12 hrs intervals. To avoid experimental errors, the females in the other groups were treated similarly. When 40 days old, each female was placed in a clean cage and paired with a sexually experienced intact male. They were checked for vaginal plugs twice daily as above. The male was removed one day after a vaginal plug was found. The females were autopsied six days after mating and the weights of ovaries and uteri were recorded along with the incidence of pregnancy. At this time, the embryos are visible as 3-4 mm swellings (Andersson and Gustafsson, 1979). The number and macroscopic appearance of corpora lutea were noted. If no vaginal plug was found before day 60, the female was autopsied as above.



## Results

### Day 30 to 40 of age; females paired with vasectomized males

Copulatory plugs were found in 28 (53%) of the 53 females kept with a vasectomized male between day 30 and 40 of age. In five of these females, two matings with the vasectomized male were recorded.

### Day 40 to 60 of age; all females paired with normal males

A much higher proportion of matings with fertile males was found in the females previously kept with vasectomized males than in females kept singly or in pairs (Table 1). Females previously kept with a male, but separated from him by a wire mesh, were intermediate.

In group D no difference in the proportion of mating was found between females which earlier had mated with a vasectomized male ( $D_M$ ) and those which had not ( $D_U$ ) (Table 1). Of the 137 mated females, 37 were visibly pregnant, two had small placental scars (one each in group A and  $D_M$ ) and three were classified as pseudopregnant (all in group  $D_U$ ). Like pregnant females, the pseudopregnant specimens had activated, large, pink corpora lutea and their ovarian weights also were similar to those of pregnant ones. The reason that they were pseudopregnant is obscure. They had all mated with a vasectomized male one to two days before mating with the normal male. The females with placental scars evidently had had corpora lutea which were active enough to initiate implantation.

Infertility in young female bank voles is due to lack of activation of the corpora lutea (Westlin and Nyholm, 1982; Westlin, 1982b). As the above five females all showed evidence of activation of the corpora lutea, they were included in the groups with fertile matings.

Fertility was similar in groups A, B and C. Group D showed a significant increase in fertility compared to those three groups (Table 1). Within group D the fertility was dependent on previous mating experience. Females which had mated with the vasectomized male (Group  $D_M$ ) showed much higher fertility after the subsequent mating with the normal male (Table 1) than the unmated ones (Group  $D_U$ ). When comparing with the other treatments, group  $D_U$  showed slightly lower fertility than groups A-C, whereas group  $D_M$  had much higher fertility than groups A-C (Table 1). Within group  $D_M$ , the few females with two recorded matings with the vasectomized male showed a higher fertility (4 out of 5) than those with only one mating (13 out of 22). If the pseudopregnant females and those with placental scars would be regarded as non-pregnant, the fertility of group  $D_M$  would still be significantly higher than that of groups A, B and C ( $p < 0.025$ , G-test of independence).

The treatments also affected the age at which mating occurred (Text-fig. 1). Animals kept with a vasectomized male mated with the normal male at a lower age than those kept singly or in groups of two females (Mann-Whitney's U-test,  $p < 0.01$  and  $p < 0.05$ ). Again females kept with a male behind a barrier were intermediate.

## Discussion

Many small rodents show estrus one to two days after introduction of an adult male. In microtines this has been reported for *M. ochrogaster* (Hasler and Conaway, 1973; Carter et al., 1980). Male-induced estrus is apparent in this study. In all groups, there was a peak in the number of matings 2-3 days after the pairing with the normal male (Text-fig. 1). After the initial peak, comparatively few matings were seen. Carter et al. (1980) reported that female *M. ochrogaster* kept with sibling males fail to reach estrus. They were not responsive to odours of the sibling males. However, if sibling male urine was applied to their nostrils, they rapidly reached estrus and mated with the sibs. The authors applied the failure to reach estrus to lack of naso-genital grooming between siblings. These findings may apply to *C. glareolus*. If the female is not mature enough to respond immediately to the male, she may develop a sibling-like relationship to the male, and be relatively unlikely to reach estrus, even when mature. Such a relationship would be broken and an estrus induced if a new male was introduced. This is seen in groups C and D, where contact with an unknown male results in induction of estrus and a high incidence of matings 2-3 days later, in spite of that they already had been in contact with adult males.

Physical or olfactory contact with a male accelerates puberty in for example *M. ochrogaster* (Carter et al., 1980; Hasler and Nalbandov, 1974), *Dicrostonyx groenlandicus* (Hasler, 1974).

In groups C and D, a much higher proportion of the females mated on days 40-44 than in groups A and B (82 and 56% vs 35 and 34%). This may be explained by a male-induced acceleration of puberty, resulting in a larger number of females being mature enough to respond with estrus to the new male.

The increased fertility in group D seems to be a direct response to mating with a vasectomized male, as it is only seen in group D<sub>M</sub>. It may be argued that the experimental design could cause the most fertile females to be selected into group D<sub>M</sub>. This is an unlikely explanation, as the proportion of females mating with the vasectomized male (D<sub>M</sub>) is very similar to the proportions mating in groups A and B.

The occurrence of sterile matings in natural populations (Westlin and Nyholm, 1982) indicates that these are a natural part of the maturation process both at puberty and in females "re-maturing" after the winter anoestrus.

The priming effect of sterile matings found here indicates that they are important in accelerating maturation of the females.

## Acknowledgements

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Table 1. Incidence of mating and fertility of matings in female *Clethrionomys glareolus*, kept in different social environment between day 30 and 40 of age, and thereafter paired with normal males. Females kept with vasectomized males are subdivided into those which mated with the vasectomized male and those which did not. Statistical method: G-test of independence

| Treatment, day 30 to 40                           | N  | Mated with normal male<br>N per cent | Fertility of matings<br>N per cent | Total fertility<br>per cent |
|---------------------------------------------------|----|--------------------------------------|------------------------------------|-----------------------------|
| A: One female per cage                            | 44 | 24 55 <sup>a,b</sup>                 | 6 25 <sup>d</sup>                  | 14                          |
| B: Two females per cage                           | 39 | 23 59 <sup>a</sup>                   | 5 22 <sup>c</sup>                  | 13                          |
| C: With normal male, separated by net             | 50 | 40 80                                | 10 25 <sup>c</sup>                 | 20                          |
| D: With vasectomized male                         | 53 | 50 94                                | 21 42 <sup>e</sup>                 | 40                          |
| D <sub>U</sub> : No mating with vasectomized male | 25 | 23 92                                | 4 17 <sup>c</sup>                  | 16                          |
| D <sub>M</sub> : Mating with vasectomized male    | 28 | 27 96                                | 17 63                              | 61                          |

<sup>a</sup>Lower than group D,  $p < 0.001$

<sup>b</sup>Lower than group C,  $p < 0.025$

<sup>c</sup>Lower than group D<sub>M</sub>,  $p < 0.01$

<sup>d</sup>Lower than group D<sub>M</sub>,  $p < 0.025$

<sup>e</sup>Higher than groups A, B, C combined,  $p < 0.05$

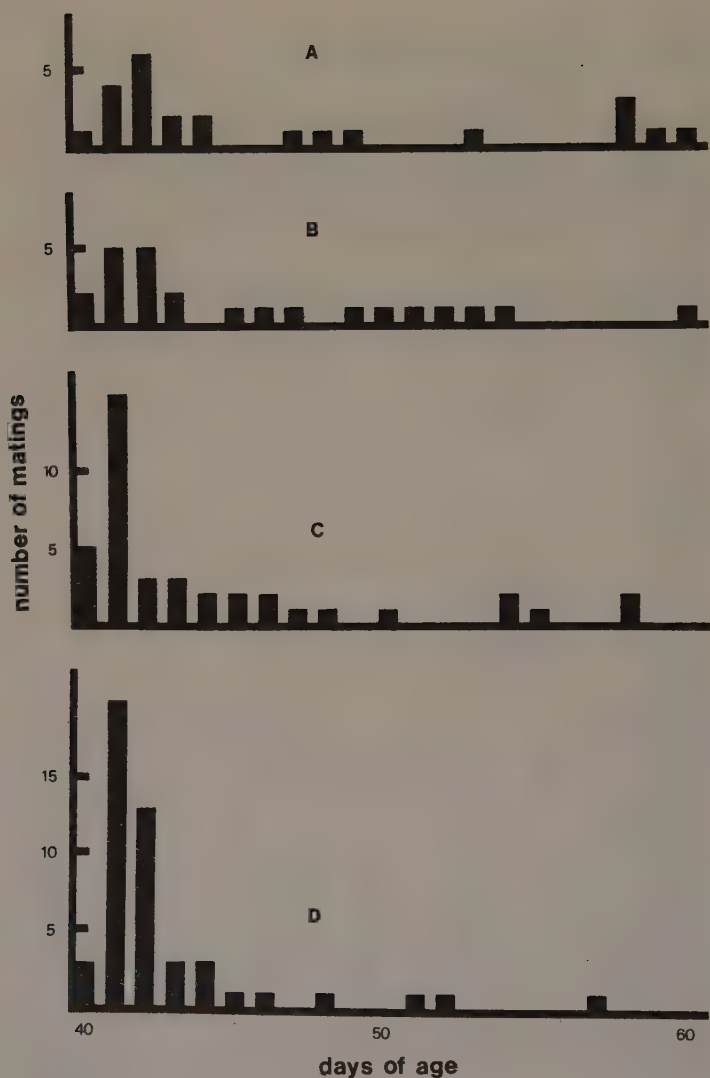


Fig. 1

Time of mating in female *Clethrionomys glareolus*, kept in different social environments between day 30 and 40 of age, and then paired with sexually experienced males. Treatments between day 30 and 40: A) kept singly, B) two females kept together, C) separated from a normal male by a net barrier, D) with a vasectomized male.



STERILE OVULATIONS IN MATURING WILD MICROTINE RODENTS -  
INSUFFICIENT COPULATORY STIMULATION OR PREGNANCY BLOCK?

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Sterile ovulations at the beginning of the breeding season occur in several microtine populations (1-9). They have been regarded as evidence for pregnancy block (10) in the wild (7,11,12). We propose another explanation: In pubertal female bank voles, Clethrionomys glareolus, or in females "re-maturing" after a winter anoestrus, the copulatory stimulus received at a normal mating is not sufficient to trigger the secretion of the complex of hormones that maintains the corpora lutea, which are crucial for early pregnancy. We show here that artificial vaginal stimulation after a normal mating results in dramatically increased fertility compared to untreated females. We suggest therefore that the sterile ovulations found in wild-caught animals should be considered as a puberty phenomenon, associated with stimulus requirements.

At the start of the breeding season, many microtine rodents ovulate several times in rapid succession before their first pregnancy. This is also seen in young of the year before their first pregnancy (1-9). These sterile ovulations result in several sets of low-activity corpora lutea in the ovaries (9). Historically, this was taken as evidence for spontaneous ovulations (1,2), but since then it has been shown that most, if not all, microtines are reflex ovulators (9,13-15).

The "sterile ovulations" have been regarded as evidence for occurrence of pregnancy block (10) in natural populations (7,11,12). We suggest another explanation, involving the mechanism for activation of the corpus luteum in these species. Microtines have a multiple intromission - multiple ejaculation copulatory pattern (16-21). In many species, e.g. Microtus agrestis, M. montanus and C. glareolus, ovulation is triggered by the first few intromissions, whereas a longer period of vaginal stimulation is needed to activate the corpora lutea (15,22,23).

We propose that in pubertal females or in females "re-maturing" after a winter anoestrus, the amount of copulatory stimulation received at a normal mating is not sufficient to trigger a prolactin release strong enough to fully activate the corpora lutea, and that puberty is associated with a lowering of stimulus requirements at mating. The evidence for this comes from a series of laboratory experiments on C. glareolus.

Young female C. glareolus frequently fail to become pregnant at their first matings. This occurs both when the stud male is left with the female (9) and when the stud male is removed and the female kept singly (24). The corpora lutea of these females strongly resemble those seen in

wild-caught females having experienced sterile ovulations before capture (9). Treatment with progesterone or prolactin results in complete fertility. Furthermore, prolactin treatment activates the corpora lutea (24).

Results from two experiments on bank voles are reported here:

1) The influence of age on fertility at the first mating. Females kept under standard laboratory conditions were mated at different ages and fertility was recorded.

2) The effect of additional vaginal stimulation on fertility in young females. Young females (30 days) were paired with sexually experienced males at 9 a.m. They were checked for vaginal plugs or mating behaviour every 2-3 hours. If no mating had occurred by 3 p.m., the male was removed and the test was repeated the following day. After mating, the male was left with the female over night and then removed. Four hours after a mating was discovered, half of the females were subjected to additional artificial vaginal stimulation with a vibrating plastic rod. The remaining females were left undisturbed. The females were kept singly until autopsy six days after mating.

1) The fertility at the first mating was strongly influenced by age. Fertility increased from less than 25% in females younger than 50 days to approximately 80% in 120-180 days old females (Fig. 1).

2) Artificial vaginal stimulation after the first mating increased fertility from 29% to 76% (Table 1). Corpora lutea of the non-pregnant specimens were all small, poorly vascularized and with low activity (9,24).

It appears that the infertility can be explained as a puberty phenomenon, associated with stimulus requirements at mating.

In bank voles, there are strong resemblances between "re-maturing" at the beginning of the breeding season and puberty (9), and there are good reasons to believe that the infertility has similar causes.

Thus, the occurrence of sterile matings in "re-maturing" or pubertal wild bank voles may be explained by immaturity of the mechanism activating the luteotrophic complex, rather than by pregnancy block.

The similarity between microtine species in the occurrence of sterile ovulations in natural populations (1-9) may be taken as an indication of a similar explanation for their infertility. It would be very interesting to know whether there is a similar relationship between copulatory stimulus requirements and fertility in pubertal females of other microtine species.

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Table 1. Fertility of young female bank voles after complete mating or complete mating followed by additional vaginal stimulation with a vibrating plastic rod\*

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|                                        | N  | Pregnant | With active corpora lutea |
|----------------------------------------|----|----------|---------------------------|
| Normal mating                          | 17 | 5        | 5                         |
| Normal mating + mechanical stimulation | 17 | 13       | 13                        |

---

Fertility was significantly increased by additional mechanical stimulation,  $p < 0.025$ , G-test of independence.

\*The rod was placed in the vagina for 2 sec at 15-sec intervals during a 2-min period. Five such 2-min stimulation periods were given at 10-min intervals. The stimulation regimen was based on observations of the mating behaviour of bank voles (16-21).

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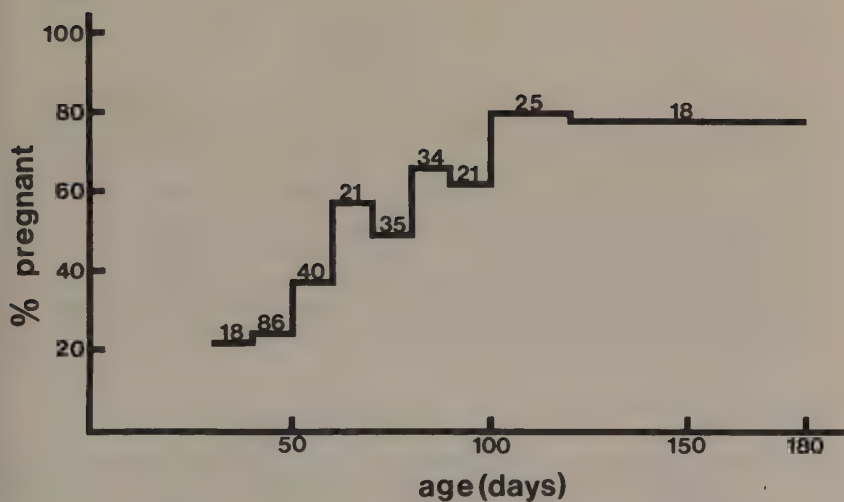


Fig. 1  
Fertility in relation to age in primiparous female bank voles.  
For each age interval, N is shown.



ACTIVITY IN THE CORPORA LUTEA RESULTING FROM STERILE MATINGS  
IN CLETHRIONOMYS GLAREOLUS, A HISTOCHEMICAL STUDY

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SUMMARY

The capacity for steroid production in the corpora lutea (CL) of bank voles was examined by enzyme histochemistry for  $3\beta$ -hydroxy-steroid dehydrogenase. The results revealed similar activity in the CL resulting from sterile matings and the CL of pregnancy two days after mating. Six days after mating, the CL of pregnancy showed a strong reaction, while there was almost no reaction in the CL from sterile matings, which were clearly regressed at this time. We suggest that 1) the corpora lutea from sterile matings produce progesterone before regression, 2) this progesterone may prime the luteotrophic complex, increasing fertility of subsequent matings.

INTRODUCTION

In several microtines, the corpora lutea (CL) developing after the initial matings of the breeding season cannot support pregnancy (for references, see Westlin & Nyholm, 1982). Although the oocytes are fertilized, the blastocysts fail to implant. Exogenous progesterone given to newly mated, young female bank voles (Clethrionomys glareolus) increases fertility to 100% (Westlin, 1982a). Prolactin, which in this species is an important part of the luteotrophic complex, also increases fertility when given to newly mated, young females (Westlin, 1982a). The CL resulting from sterile matings are short-lived and appear non-functional compared with those of pregnancy. They are smaller, less vascularized and the cells seem poorly luteinized (Westlin & Nyholm, 1982). There is, however, a short period after mating (2-3 days) when the histology of the CL resulting from sterile matings and the CL of pregnancy is rather similar. It is possible that sterile matings have a priming effect on the female during puberty, or after a winter anoestrus of the adult. Matings would then stimulate the luteotrophic complex until the threshold necessary for activating the CL is reached and pregnancy ensues. In the laboratory, young female bank voles mated several times before becoming pregnant (Westlin & Nyholm, 1982). The number of sterile matings varied between the individuals (some of them mated up to eight times). This study deals with whether the CL resulting from sterile matings have the capacity of steroid production before they start to regress. If so, this would support the theory that sterile matings have a priming effect on females at the onset of breeding.



## MATERIALS AND METHODS

### Animals and treatment

We used bank voles from the laboratory colony maintained at the Department of Zoology, University of Lund, Sweden, which have been described by Gustafsson, Andersson & Westlin (1980). The animals were kept in standard, plastic mouse cages (40 cm x 20 cm x 15 cm) with a wire mesh cover, at a photoperiod of 18 h light/6 h dark and a temperature of 22 - 5°C. Wood shavings were used as bedding. The animals were allowed water and commercial mouse food (Astra-Ewos R3) ad libitum, supplemented twice a week with rabbit and guinea-pig pellets (Astra-Ewos).

After weaning at 18 days, females were kept in single sex groups of 5 until 50 days old, when the experiments started. Each female was then paired with an adult, sexually experienced male and checked for mating every third hour. A copulatory plug was taken as evidence of mating. The male was removed the day after mating. Females not mated within 25 days were excluded.

Two days after mating, the females were unilaterally ovariectomized under ether anaesthesia and the removed ovaries were frozen immediately at -70°C. They were stored in a deep freezer at the same temperature.

Six days after mating, the females were autopsied and checked for pregnancy. Implantation occurs 4.5 days after mating in this species and six days after mating, the embryos are clearly visible as uterine swellings (Andersson & Gustafsson, 1979). The remaining ovary was dissected out and treated as described above. To avoid differences in histochemical reactions due to processing, all ovaries were treated simultaneously.

### Histochemical technique

Enzyme-histochemistry for detecting 3 $\beta$ -hydroxy-steroid dehydrogenase (3 $\beta$ -HSD), an enzyme occurring early in the pathway for steroidhormone biosynthesis (Samuels, 1960; Rubin, Deane & Hamilton, 1963; Deane & Rubin, 1965), was carried out in the following way: The ovaries were sectioned at 12  $\mu$ m in a cryostat set at -25°C using a cooled knife. The sections were attached to clean dry microscope slides by momentary thawing and stored in a covered container in a deep-freezer.

On the following day, the sections were stained to demonstrate activity of 3 $\beta$ -HSD. The method was modified from that described by Baillie, Calman, Ferguson & Hart (1966) and van den Hurk (1973). Before incubation, the tissue preparations were dried at room temperature for two minutes and then kept in xylol for two minutes to remove lipids. The tissue was dried well in air and then incubated at 37°C for 30 minutes. The medium is given in Table 1. The control medium was similar, except that it lacked epiandrosterone. After incubation the preparations were fixed in 5% formaldehyde for five minutes, rinsed in distilled water, and mounted in glycerol-gelatine-medium. The sections were examined anonymously by light-microscopy.

## RESULTS

Six of the thirteen mated females became pregnant. The proportion of pregnancy agreed well with earlier experiments (Westlin & Nyholm, 1982; Westlin, 1982a). Dissection showed that the embryos were all normal and that no resorptions had occurred. The number of CL in the pregnant animals corresponded exactly with the number of embryos. Ovariectomy did not reduce fertility. In the bank vole, it is possible to determine the success of mating from the appearance of CL already two days after mating. This was determined at ovariectomy and was confirmed in all cases at autopsy.

### Histology

The CL of pregnancy, both two and six days after mating, were large and richly vascularized and the cells were well luteinized. The CL of non-pregnant females two days after mating showed the histological picture described elsewhere (Westlin & Nyholm, 1982; Westlin, 1982a). They were small and poorly vascularized and the cells did not appear as luteinized as those of CL from pregnant animals. Six days after mating, the CL resulting from sterile matings were regressed. Their sizes were decreased, the vascularization had disappeared and the cells had pyknotic nuclei (Westlin & Nyholm, 1982; Westlin, 1982a).

### Histochemistry

Two days after mating, the CL resulting from sterile matings showed a clear reaction for  $3\beta$ -hydroxy-steroid dehydrogenase (Fig. 1). However, six days after mating, i.e. 1.5 days after the time when implantation occurs, these CL were distinctly regressed and showed almost no reaction for the enzyme (Fig. 3). In the pregnant animals two days after mating, the CL showed a clear reaction for  $3\beta$ -HSD (Fig. 2). There were no visible differences between these CL and the CL resulting from sterile matings as regards the strength of the histochemical reaction. Six days after mating, the CL of pregnancy showed an even stronger reaction for the enzyme (Fig. 4) than on day two.

## DISCUSSION

During puberty or at the beginning of the breeding season, many small rodents experience one or more sterile ovulations, forming one or more sets of short-lived CL before the first pregnancy. This is true for reflex ovulators like Clethrionomys rutilus and Microtus oeconomus ratticeps (Hoyte, 1955), the field mouse, M. californicus (Greenwald, 1956), the pine vole, M. pinetorum (Kirkpatrick & Valentine, 1970), M. pennsylvanicus (Mallory & Clulow, 1977), M. ochrogaster (Rose & Gaines, 1978), M. agrestis (Brambell & Hall, 1939; Westlin, 1982b) and C. rufocanus (Westlin, op. cit.), as well as for

spontaneous ovulators like Peromyscus maniculatus and P. boyleyi (Jameson, 1953), P. eremicus, Mesocricetus auratus, Rattus conatus, the muskrat, Ondatra zibethica (Asdell, 1964) and several others.

For spontaneous ovulators it is not known whether matings take place before the sterile ovulations, while for reflex ovulators, the sterile ovulations result from mating (Westlin & Nyholm, 1982; Westlin, 1982a). In all the species investigated so far, mating is required in order to activate the CL, regardless of the ovulatory stimulus. In Clethrionomys glareolus, oocytes are fertilized at these matings, but the short-lived CL do not produce enough progesterone and the fertilized eggs are lost at the time of implantation or before. Treatment of young, mated female Clethrionomys glareolus with progesterone results in full fertility, as does treatment with prolactin, which acts by activating the CL (Westlin, 1982a). Two days after mating, the enzyme reaction was as strong in CL of non-pregnant voles as in those of pregnant ones. This suggests that the CL resulting from sterile matings at this time produce progesterone.

Female C. glareolus which have experienced a sterile ovulation are more fertile than those which have not (Westlin & Gustafsson, MS). Thus, it appears that a sterile ovulation in some way acts to prime the animal and increase the fertility of subsequent mating.

That the short-lived CL resulting from sterile matings are likely to produce progesterone, suggests the following priming mechanism: The CL are activated by prolactin (Westlin, 1982a), and progesterone produced by the short-lived CL may prime some part of the control system for prolactin-release thus increasing the support for the CL which will result from subsequent mating. There are several possible sites for this priming: 1) the pituitary; 2) the hypothalamus; 3) the vagina, acting to increase the sensitivity of its receptors for copulatory stimulation. It is also possible that for the first pregnancy to occur, the neural pathways leading from the vagina to the hypothalamus need a preceding mating to become fully developed. These possibilities are being investigated.

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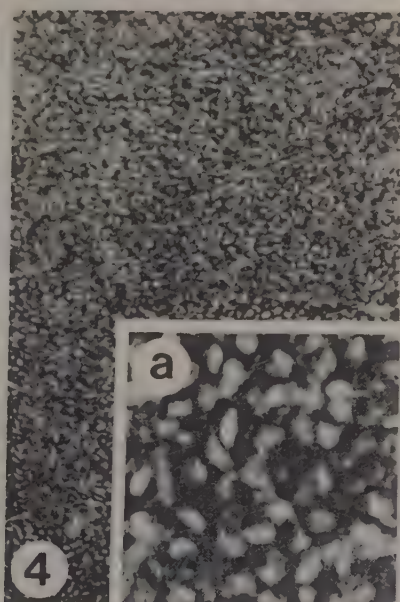
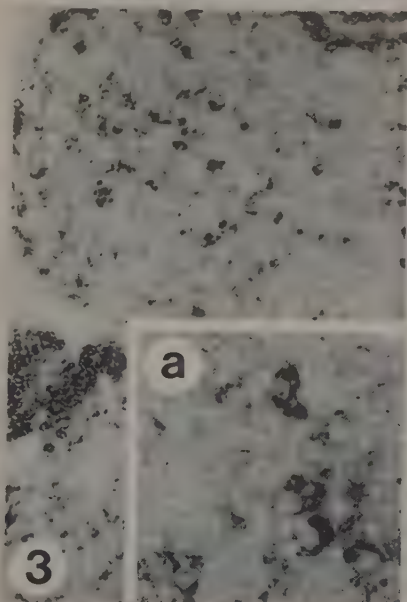
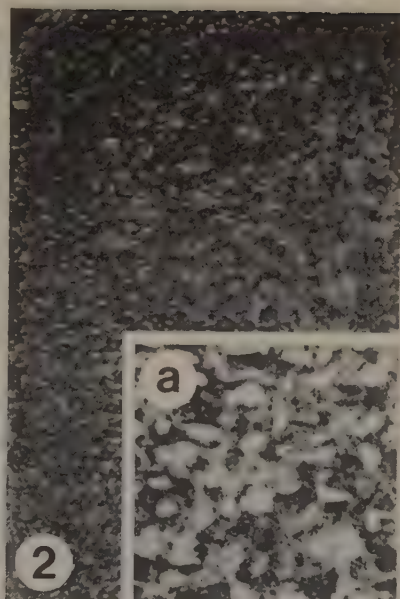
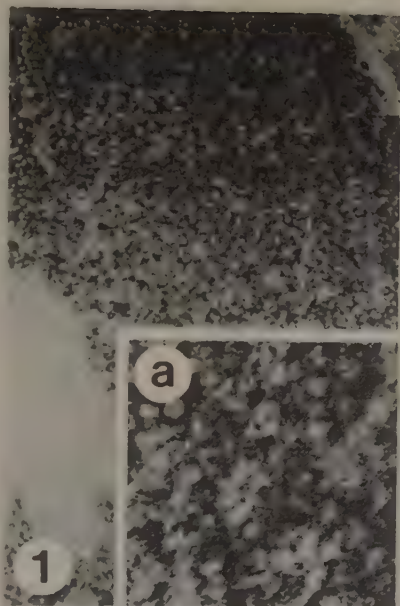
Table 1. Composition of medium for demonstrating the activity of  $3\beta$ -hydroxysteroid dehydrogenase

|                                                    | Amount |
|----------------------------------------------------|--------|
| Phosphate buffer 0.1 M, pH 7.4 .....               | 20 ml  |
| Dimethylformamide (BDH Chemicals Ltd) .....        | 2 ml   |
| Epiandrosterone (United States Bioch. Corp.) ..... | 2 mg   |
| Nitro blue tetrazolium (Merck) .....               | 4 mg   |
| NAD (Merck) .....                                  | 10 mg  |

# PLATE 1

Corpora lutea from one non-pregnant (Figs. 1 and 3) and one pregnant (Figs. 2 and 4) bank vole. x 94. Two days after mating the reaction for  $3\beta$ -hydroxysteroid dehydrogenase (seen in the cytoplasm as dark spots) is of similar strength in the non-pregnant (Fig. 1a) and the pregnant (Fig. 2a) animal. x 370. Six days after mating the CL resulting from a sterile mating show a very low reaction for the enzyme (Fig. 3a), while at that time, the reaction has increased in the CL from the pregnant specimen (Fig. 4a). x 370.

PLATE 1







DOPAMINE AS A POSSIBLE PROLACTIN INHIBITING FACTOR (PIF)  
IN FEMALE BANK VOLES, CLETHRIONOMYS GLAREOLUS

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**Summary.** Fertility in young primiparous female bank voles was markedly improved by daily injections after mating of 0.2 mg haloperidol, a dopamine antagonist. This treatment increased the proportion of healthy corpora lutea to 100 %, compared with 27 % in controls receiving vehicle only. The activation of the luteotrophic complex by a dopamine antagonist suggests that dopamine acts as a prolactin inhibiting factor (PIF) in female bank voles.

### Introduction

Microtines are induced ovulators (Breed, 1967; Clarke, Clulow & Greig, 1970; Westlin & Nyholm, 1982) and all so far investigated initiate their breeding season with a series of matings that do not result in pregnancy (for references, see Westlin & Nyholm, 1982). The corpora lutea (CL) of these sterile matings regress quickly (Westlin & Nyholm, 1982) due to inadequate stimulation of the luteotrophic complex (Westlin, 1982). Coitus is known to activate this complex in female voles (Milligan, 1975) and the activation can be increased by a) additional artificial vaginal stimulation (Westlin & Gustafsson, 1983a), b) earlier sexual experience (Westlin & Gustafsson, 1983b), or prolactin treatment after mating (Westlin, 1982).

Dopamine is known to act as a prolactin inhibiting factor (PIF) in rats (VanMaanen & Smelik, 1968; MacLeod, 1969; Birge, Jacobs, Hammer & Daughaday, 1970; MacLeod, Fontham & Lehmyer, 1970; Shaar & Clemens, 1974).

The present study was undertaken to examine whether activation of the luteotrophic complex in newly mated female bank voles could be increased by treatment with a dopamine-antagonist, haloperidol.

### Materials and Methods

**Animals.** The bank voles, Clethrionomys glareolus, were bred in the Department of Zoology, University of Lund, and maintained as described by Gustafsson, Andersson and Westlin (1980).

Females were weaned at 18 days of age and kept in single-sex groups. When 30 days old, the females were kept singly for 2 days and then subjected to mating tests. A male, 2 months old and sexually experienced, was introduced into the female's cage at 09:00 h, and the female was checked every 2 h for a copulatory plug or for mating behaviour.

After mating, the male was left with the female until the following morning. If no mating occurred during the day, the male was removed and the test was repeated the following day. Females which had not mated when 2 months old were discarded.

Injections. All injections were given subcutaneously beneath the skin at the back of the head without anaesthesia. Haloperidol (Haldol<sup>R</sup>, AB LEO, Helsingborg, Sweden) was dissolved in a minimal amount of acetic acid (100 %) and diluted with glucose (5.5 %) to a concentration of 1 mg haloperidol/ml glucose. The dose given was 0.1 mg haloperidol in 0.1 ml vehicle/20 g body weight. Control females were injected with glucose only.

Experimental methods. After mating the females were randomly assigned to haloperidol treatment or control treatment. The control animals were given glucose only every 12 h for 5.5 days (the total number of injections was 11) starting 12 h after mating. In a similar way the experimental animals were given haloperidol every 12 h for 5.5 days. All females were killed 6 days after mating, a time when embryos are easily seen in the uteri as 3-4 mm swellings (Andersson & Gustafsson, 1979). At autopsy, ovaries and uteri were examined macroscopically for the presence of large pink CL and embryos respectively, dissected out, fixed in Bouin's fluid, and routinely treated for histology. The ovaries were serially sectioned at 7  $\mu$ m and stained with a tetrachrome combination according to Borg, Andersson & Meurling (1978). Histologically, two types of CL were distinguished: (1) large CL with large well luteinized cells and richly vascularized, and (2) small CL with typical signs of regression (see Westlin & Nyholm, 1982).

## Results and Discussion

No relationship between age at mating and fertility was seen. Following mating, all the females possessed CL, confirming that no infertile matings were caused by failure of ovulation.

Only 27 % (4 out of 15) of the control females became pregnant, a normal fertility rate in primiparous bank voles under laboratory conditions (Westlin, 1982). The non-pregnant females all had regressed CL (Table 1). In contrast, haloperidol treatment after mating resulted in pregnancy in 80 % (12 out of 15) of the females (Table 1) and activated corpora lutea in all females. The non-pregnant females had large pink CL but no embryos. Assuming that haloperidol treatment increased prolactin levels, pseudo-pregnancy is not unexpected since an abnormally high level of prolactin can cause suppression of fertility (Ben-David, Danon & Sulman, 1971).

It has been shown by several researchers (VanMaanen & Smelik, 1968; MacLeod, 1969; Birge, Jacobs, Hammer & Daughaday, 1970; MacLeod, Fontham & Lehmeyer, 1970; Shaar & Clemens, 1974) that dopamine inhibits prolactin release in e.g. rats.

There is a tuberoinfundibular dopamine neuron system which ends in the median eminence (Fuxe & Hökfelt, 1969, 1970; Shute, 1970). This system may be involved in the transmission of information from the brain to the pituitary. The dopamine neurons may act to control the release of PIF from the secretory neurons (McCann, 1970; Fuxe & Hökfelt, 1969, 1970).

Bromocriptine (a dopamine agonist) given to newly mated female rats resulted in inhibited implantation due to lack of luteotrophic support (Müller, Bauknecht & Siebers, 1980), a situation similar to that after sterile matings in the bank vole (Westlin, 1982). Since haloperidol is a dopamine antagonist and thus suppresses the action of dopamine by occupying the receptor sites for dopamine and by lacking intrinsic activity, I suggest that dopamine acts as a prolactin inhibiting factor in female bank voles.

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Table 1. The effect (at day 6) of twice daily injections of haloperidol, a dopamine antagonist, on fertility and the function of corpora lutea (CL) in mated, primiparous female bank voles.

| No. of females | Treatment   | No. of females      |                     |              |
|----------------|-------------|---------------------|---------------------|--------------|
|                |             | With degenerated CL | With healthy CL (%) | Pregnant (%) |
| 15             | haloperidol | 0                   | 15(100)             | 12(80)       |
| 15             | glucose     | 11                  | 4(27)               | 4(27)        |

Fertility was significantly increased in the haloperidol-treated females ( $p < 0.01$ ; G-test of independence).

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